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Center for Air Environment Studies

The Pennsylvania State University

FINAL PROGRESS REPORT

to

Department of the Air Force
Air Force Office of Scientific Research
Building 410
Bolling Air Force Base, DC 20332

for

Grant No. ~~AFOSR-2767~~ AFOSR-75-2767

Title: INFLUENCE OF ALTERED GASEOUS ENVIRONMENTS ON LUNG METABOLISM

Project Period: July 1, 1974 to August 31, 1976

Submitted by: Rodney A. Rhoades (Ph.D.)
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Center for Air Environment Studies
252 Penske Laboratory
University Park, PA 16802

September, 1976

CAES

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- Research on effects of air pollutants on trees, food, and fiber crops; predisposition to attack by other pathogens; and economic loss through damage to plants.
- Studies of small particle behavior, particle detectors, and particle collection devices.
- Development of high accuracy, low cost, mobile, analysis equipment for routine sampling of ambient air.
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- Studies of combustion processes leading to lower contaminant emissions.
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Isolated lungs were perfused 1.5 hours with a medium of washed bovine red cells resuspended to a 15 percent hematocrit with Krebs-Henseleit bicarbonate buffer containing 5 gram percent albumin. Substrate concentrations were 6 millimolar glucose and 1 millimolar palmitate. Data from altered carbon dioxide tension experiments indicate that prior exposure to high carbon dioxide tension markedly alters the lung's ability to maintain synthesis of cellular constituents from glucose. In a second series of experiments acute

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effects of hyperoxia on lung metabolism were examined. Lactate production increased and pyruvate decreased. Data from a series of tests of hypoxia on cyclic nucleotides indicate that hypoxia has a selective action on lung cAMP and nutritional stress potnetiates a different effect than hypoxia. Other data indicate that a 24 hour hypoxic-hypercapnia exposure drastically alters lung weight, glycolysis and lipid synthesis in the lung.

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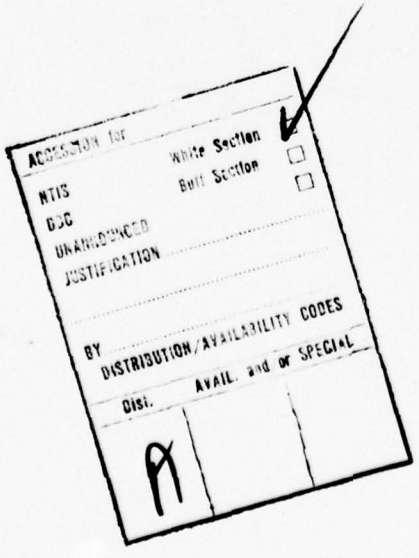

I. ABSTRACT

Isolated lungs were perfused 1.5h with a medium of washed bovine red cells resuspended to a 15% Hct with Krebs-Henseleit bicarbonate buffer containing 5g% albumin. Substrate concentrations were 6mM glucose and 1mM palmitate. Lungs were ventilated 100 cycles/min and controls received 21% O_2 - 5% CO_2. In the first series of experiments effects of altered CO_2 tension were examined. For hypocapnia, lungs from normal rats were removed, perfused and ventilated with 21% O_2 - 3% CO_2 (balance N_2) for 1.5h. For hypercapnia, lungs from normal rats and rats exposed to 24h hypercapnia (12% CO_2) were removed, perfused and ventilated with 21% O_2 - 10% CO_2 (balance N_2) for 1.5h. Control lungs for all groups were ventilated 21% O_2 - 5% CO_2 (balance N_2). Exposure of normal rats lungs to 1.5h in vitro hypocapnia (PCO_2 = 22.8mmHg ± 0.3SE) significantly ($P < 0.05$) increased lactate (42%) and pyruvate (20%) levels, with no effect on U - ^{14}C glucose incorporation into lung lipids or oxidation to $^{14}CO_2$. Normal rat lungs removed and made hypercapnic in vitro (PCO_2 = 65mmHg ± 1.6SE) did not alter U - ^{14}C glucose incorporation into lung lipids, glucose oxidation to $^{14}CO_2$, lactate production, but did result in a significant 26% decrease in pyruvate levels. In contrast to normal lungs, prior exposure to hypercapnia (12% CO_2) for 24h and subsequent perfusion under hypercapnic conditions significantly depressed glucose uptake (47%) labelled glucose incorporation into phospholipids (29%), glucose oxidation to $^{14}CO_2$ (39%), lactate production (34%) and pyruvate levels (48%). These data indicate that prior exposure to high CO_2 tension markedly alters the lung's ability to maintain synthesis of cellular constituents from glucose.

In a second series of experiments, acute effects (24h) of hyperoxia on lung metabolism were examined. Rats were first exposed to 100% O_2 for 24h and then lungs were excised and placed on the perfusion preparation.

Hyperoxic lungs were ventilated with 95% O_2 - 5% CO_2. Lactate production showed a significant ($P < 0.05$) 27% increase while pyruvate resulted in a 36% decrease. Lipid synthesis, and lung dry/wet were not significantly ($P < 0.05$) affected.

In a third set of experiments acute effects (24h) of hypoxia on lung cyclic nucleotides were investigated. Male Long Evans Hooded rats were exposed to a simulated altitude of 7,193 meters (23,600 feet:P_B=280mmHg) for 24h. Control animals were maintained at atmospheric pressure. Both groups received water ad libitum, but each group was food deprived for the 24h period, since food intake is substantially reduced in rats during hypoxic exposure. Cyclic nucleotides were also examined in liver tissue to evaluate comparatively with the lung. Acute 24h hypoxia resulted in a significant 76% decrease in lung adenosine 3', 5' - monophosphate (cAMP). Lung guanosine 3', 5' - monophosphate (cGMP) was unaltered as well as liver cAMP and cGMP following 24h hypoxia. In contrast rats fasted for 72h showed a significant 3 fold increase in lung cAMP (picomoles/mg tissue) and an 8 fold increase in liver cAMP. Tissue cGMP for both tissue was unchanged by a 72h fast. These data indicate 1) that acute hypoxic stress has a selective action on lung cAMP and 2) nutritional stress potentiates a different effect on cAMP in lung and liver than hypoxic stress.

In a fourth series of experiments the effect of hypoxia on phospholipid fatty acid synthesis was examined in the isolated perfused lung. The lung has the ability to esterify exogenous free fatty acid into phospholipids as well as to synthesize phospholipid fatty acid (PLFA) endogenously from glucose. The effect of hypoxia on the relative contribution of preformed fatty acid versus endogenous synthesis of PLFA was assessed by comparing the ratio of 9, 10-H^3-palmitate to U-^{14}C-glucose incorporated into PLFA of perfused lungs.

Palmitate incorporated into PLFA averaged 3562 ± 225 nmoles/g dry lung/hr (±SE) compared to 408.3 ± 48.3 nmoles/g dry lung/hr for glucose yielding a palmitate:glucose ratio of 8.47 ± 0.84; indicating 8 moles of fatty acids are converted into PLFA for every mole of glucose. Hypoxia (lungs ventilated with 5% O_2 - 5% CO_2 compared to lungs ventilated with 21% O_2 - 5% CO_2) did not alter the relative contribution of palmitate and glucose incorporation into PLFA (ratio = 8.71 ± 0.47). Corticosterone (10^{-5}M in the perfusion medium) did not significantly ($P>0.05$) change the ratio (9.34 ± 0.8). These data indicate that 1) lung PLFA are synthesized primarily from esterification of preformed fatty acids and endogenous synthesis, either de novo or by chain elongation, is of minor significance; thus emphasizing the importance of fatty acid uptake by lung 2) PLFA synthesis is not immediately affected by hypoxia and corticosterone.

In the last series of experiments the effect of 24h hypoxia-hypercapnia (9% O_2 - 12% CO_2) exposure on lung metabolism was examined. Rats were first exposed for 24h to the altered gas mixture and lungs were subsequently removed and placed in an isolated perfused organ preparation. A 24h exposure to the hypoxic-hypercapnic gas mixture significantly increased lung wet and dry weights. Glucose uptake (a-v difference) and lactate production by the isolated perfused lung were also markedly accelerated by 67 percent and 30 percent, respectively. U-^{14}C-glucose oxidation to CO_2 was not altered but incorporation into lipids showed a 90 percent increase in the IPL following 24h exposure. 9, 10-^{3}H palmitate incorporation showed a 57 percent increase in neutral lipids plus free fatty acids but not in phospholipids. These data indicate that a 24h acute hypoxic-hypercapnic exposure drastically alters lung weight, body weight, and glycolysis and lipid synthesis in the lung.

A. A Perfused lung preparation for studying altered gaseous environments

Because of its architectural design the lung becomes a primary target organ to environmental insults. However, fundamental knowledge regarding effects of environmental pollutants on the functional processes at the tissue level is lacking. The use of the isolated perfused lung (IPL) preparation seems particularly well suited to investigate early toxic effects of such pollutants in an attempt to gain insight into underlying mechanisms which lead to lung injury.

The design of our IPL preparation is one that is as physiologic as possible and yet permits a wide range of flexibility in experimental design.

METHODS

Male Long-Evans hooded rats were heparinized (1 unit/g body weight) 20 minutes before they were sacrificed. Animals were then anesthetized with an intraperitoneal injection of sodium pentobarbital (6 mg/Kg) and exsanguinated via a carotid artery. The trachea was cannulated, lungs were removed from the chest and the left atrium removed. Lungs were kept inflated at all times. The pulmonary cannula consisting of polyethylene tubing (2.0 mm id, 2.8 mm od; 3 M Co., St. Paul, Minn.) was filled with Krebs-Henseleit bicarbonate buffer (KHB) and inserted into the pulmonary artery. The atrioventricular valves were ligated. Lungs were then placed in an organ chamber (500 ml Erlenmyer flask with a blown side arm for sample collection) which housed both the lung and perfusion medium. A schematic of the IPL is shown in Figure 1, and consists of four organ chambers housed in a temperature controlled lucite box with a circulating fan. Temperature was maintained at 37°C. The apparatus permitted simultaneous perfusion of three lungs and a blank. The blank consisted of circulated perfusate without a lung and was used to subtract metabolite contribution from the medium.

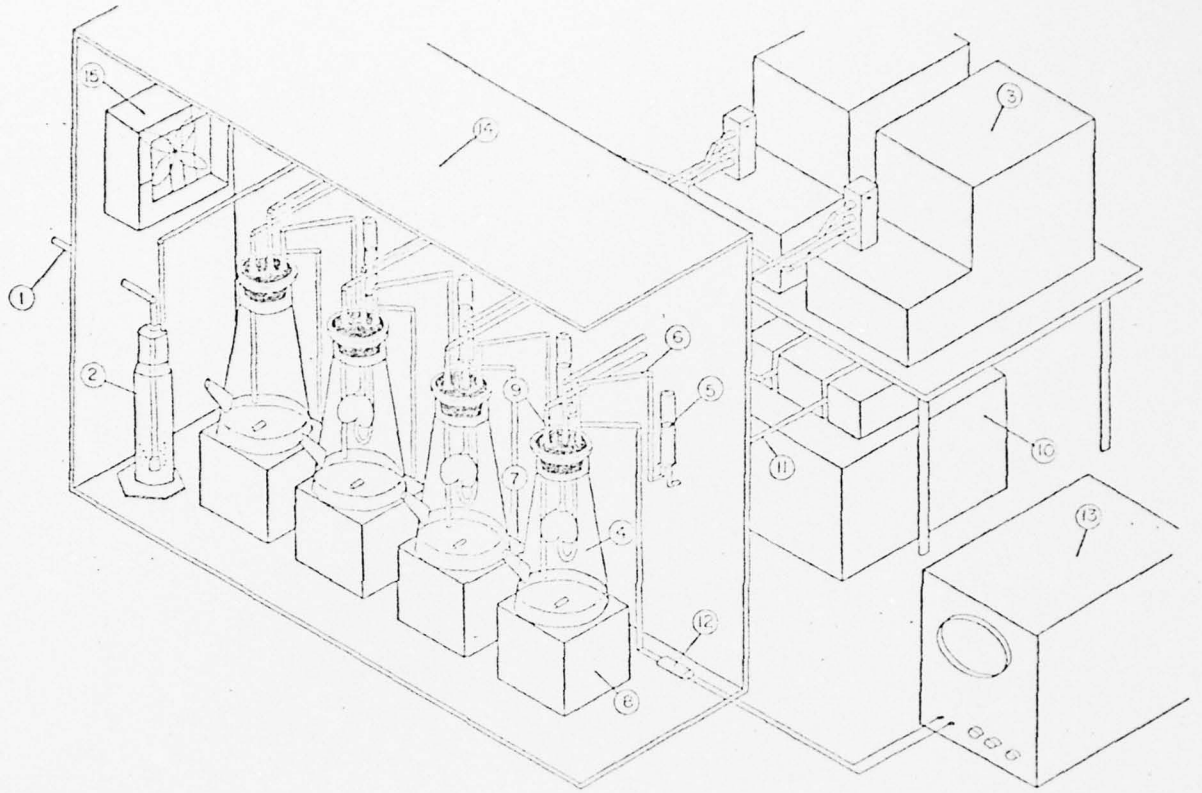

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Figure 1. SCHEMATIC DIAGRAM OF THE PERFUSION APPARATUS

Lungs were ventilated with a Harvard positive pressure ventilation pump (Model #680) at a frequency of 50 cycles/min. Ventilation was arranged such that lungs could be ventilated with any desired gas composition with concomitant collection of expired gas. Positive end expiratory pressure (PEEP) was maintained at 3 cm H_2O. The perfusion medium consisted of washed bovine red blood cells resuspended to a 15% hematocrit in KHB buffer containing 6g% dialyzed Pentex bovine serum albumin (Miles Laboratories, Inc. IL). Substrates included 6 mM glucose and 0.4 mM palmitate. The 6% BSA-KHB buffer was filtered twice through millipore filters (0.8μ and 0.45μ, respectively) washed RBC's were added, pH adjusted with 0.8 M Na carbonate, and 70 ml of perfusate placed in each organ chamber. Preparation of the BSA solution, washing of RBC, and binding of palmitate have been described previously (1). The perfusion medium was circulated with a Harvard Peristaltic Pump (10 ml/min) and passed through a specially designed chamber to dampen pulmonary pressure and remove emboli before entering the lung. Pulmonary pressure was monitored with a Statham pressure transducer (P23BB). Perfusate PO_2, PCO_2 and pH were measured with a Corning blood gas analyzer (#165). All lungs were perfused for 1.5h. Procedures for measuring lactic acid, pyruvate isolating and counting lung lipids, and counting CO_2 have been described in detail elsewhere (2, 3).

RESULTS

As seen in Table 1, blood gases and pH remained stable throughout the 1.5h perfusion period when lungs were ventilated with 95% O_2 - 5% CO_2. Although not shown, lungs ventilated with 21% O_2 - 5% CO_2 also maintained stable blood gases and pH. Figure 2 shows pulmonary pressure at various time intervals during the perfusion period. In panel A, is a normal pulmonary pressure curve from a stable IPL with a mean pressure of 13.0 mm Hg. In

Table 1. Blood gases from perfused lungs ventilated with 95% O_2 - 5% CO_2†

MEAN ± S.E.

N = 6

MEASUREMENT	PERFUSION TIME 0	PERFUSION TIME 90
pH	7.396 ± 0.004	7.398 ± 0.021
P_{O_2} (mm Hg)	481.9 ± 24.5	437 ± 18.5
P_{CO_2} (mm Hg)	37.9 ± 0.9	36.9 ± 0.9
HCO_3^- (mM)	23.3 ± 0.6	23.8 ± 0.8
TOTAL CO_2 (mM)	24.2 ± 0.6	24.8 ± 0.8
BASE EXCESS (mEq/l)	-0.8 ± 0.6	0.01 ± 0.8

† Values are means ± S.E. lungs were perfused for 1.5h at a flow rate of ~10ml/min and were ventilated 75 cycles/min.

Figure 2. Pulmonary pressure from normal lung (Panel A) and from unstable perfused lung (Panel B).

panel B, is a pulmonary pressure curve from an unstable preparation. In the absence of red cells in the medium, over half of the perfused lungs exhibited this type of phenomenon with marked edema (weight gain over 5%). In our hands, the presence of washed cells in the medium appears to maintain capillary patency and less than 1% weight gain occurs; no lungs are discarded because of edema.

Pulmonary pressure in the perfused lung can serve as a sensitive indicator of environmental stresses on the pulmonary vasculature. In a previous study (3) perfused lungs made hypoxic by ventilating them with 5% O_2 - 5% CO_2 showed a significant 36% increase in mean pulmonary pressure.

Figure 3, shows the results of glucose incorporation into lung lipids with time. For the 1.5h perfusion period, glucose incorporation was linear with time indicating tissue viability. Although not shown, over 65% of the total lipid radioactivity appeared in the phospholipid fraction. Glucose uptake (arteriovenous difference) and lactate production have also been shown to be linear with time (1). Table 2 shows the acute effects of altered CO_2 tension on lactate and pyruvate production. Perfused lungs made hypocapnic by ventilating them with 21% O_2 - 3% O_2 showed a significant 42% increase in lactate production while perfused lungs made hypercapnic (21% O_2-10% CO_2) did not appreciably alter lactate production. Pyruvate production appeared to be inversely affected by CO_2 tensions (i.e., increased with hypocapnia and vice versa) with a significant 35% increase in lactate to pyruvate (L/P) ratio in hypercapnic lungs. Under normal conditions some lactate is formed from pyruvate yielding a L/P ratio in the range of 10-14 (4). As seen in Table 2, under hypocapnia both lactate and pyruvate increased somewhat proportionally whereas in the hypercapnic condition lactate was essentially unchanged and pyruvate decreased. The L/P ratio not only gives insight into the cytoplasmic redox state but also could potentially serve to differentiate between hypocapnic and hypercapnic conditions at the tissue level.

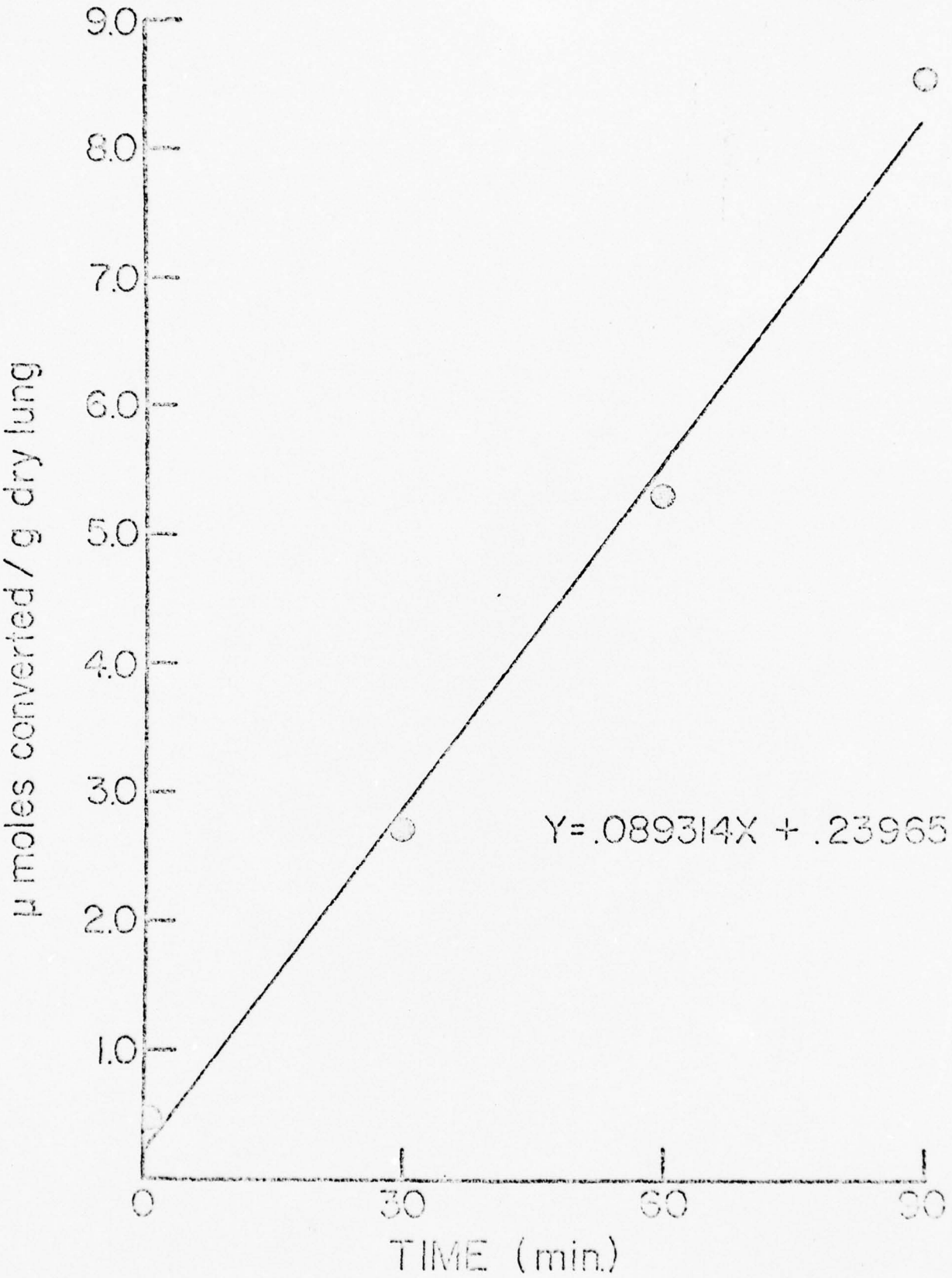

Figure 3. ^{14}C-glucose incorporation into lung lipids with time.

Table 2. Influence of altered CO_2 tension on lactate and pyruvate production in the isolated perfused lung†

Metabolite	Control (PCO_2=34mmHg)	Hypocapnia (PCO_2=22mmHg)	Hypercapnia (PCO_2=65mmHg)
Lactate[a]	96.1±10.0	136.6±10.9*	102.6±6.7
Pyruvate[a]	9.5±0.5	11.4*	7.0±0.5*
L/P	10.2±1.1	12.0±0.8	13.9±1.2*

†Values are means ±SE. Lungs were perfused for 1.5 h at a flow rate of 10 ml/min.

[a]Values denote levels in circulating medium and are expressed as μ moles/g dry lung/h (N=7/group).

*Statistically significant from control ($P<0.05$).

In summary, the isolated perfused lung serves as a physiologic model to study quantitative effects of altered gaseous environments on lung metabolism and offers several distinct advantages: 1) the lung remains an intact organ which permits the study of functional properties associated with metabolism which may not exist when isolated components are investigated. 2) Blood flow, substrate concentration, ventilation and blood gases can be controlled. 3) Transfer of extracellular nutrients from perfusate to lung is physiologic, i.e., capillary circulation.

The IPL apparatus consists of four perfusion flasks housed in a temperature controlled lucite box with a circulating fan. Lungs are ventilated by a positive pressure ventilation pump. The ventilation is arranged so that the lung can be ventilated with any desired gas composition with concomitant collection of expired gases. The perfusion medium is circulated at 10 ml/min with a peristaltic blood pump, and passes through a specially designed chamber to dampen pulmonary pressure and remove emboli. The perfusion medium presently used in our experiments consists of washed bovine red blood cells resuspended to a 15% hematocrit with Krebs-Henseleit bicarbonate buffer containing 6g% dialyzed Pentex bovine serum albumin. Circulating substrates include 6 mM glucose and 0.4 mM palmitate. pH is adjusted to 7.4 with 0.8 M Na carbonate. Lungs perfused for 1.5h with this apparatus maintain viability, show little edema, maintain blood gases, and show linear incorporation of labeled glucose into lung lipids. Perfused lungs made hypocapnic show a significant ($P<0.05$) rise in lactate and pyruvate while perfused lungs made hypercapnic show a significant decrease in pyruvate with no change in lactate.

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B. Effect of acute hyperoxia (24h) on lung metabolism

The purpose of this study was to examine the effect of acute hyperoxia on substrate metabolism. Male Long Evans Hooded rats were exposed to 100% O_2 for 24h. Following the exposure, lungs were removed and placed on an isolated perfused organ apparatus (figure 1). All perfusions were carried out at 37°C. Lungs were perfused for 1.5 hours with a medium containing washed bovine red blood cells resuspended to a 15% hematocrit with Krebs Henseleit bicarbonate buffer containing 5g% Pentex bovine serum albumin. Glucose and palmitate concentrations were 6mM and 1mM, respectively. pH was adjusted to 7.4 with 0.8M sodium carbonate. Substrate uptake was calculated as a product of initial and final concentration differences and perfusate volume. The amount of glucose degraded by blood cells was accounted for by circulating the medium in one set-up (blank) without a lung and measuring the substrate concentration changes attributed to the blood. All lungs were ventilated 100 cycles/min at tidal volume of 2.0ml. Control lungs were ventilated with 21% O_2 - 5% CO_2. Hyperoxic lungs were ventilated with 95% O_2 - 5% CO_2. Ten µCi of glucose-U-^{14}C (specific activity of 15 mCi/mmole) was added as a single pulse. From table 1, P_{CO_2} and pH were not significantly different from control lungs. Although glucose uptake in the perfused lungs was unchanged following 24h hyperoxia, lactate showed a significant ($P < 0.05$) 17% increase in lactate production and a 41% decrease in pyruvate levels. Accordingly, lactate/pyruvate ratio showed a significant 100% increase in the 24h hyperoxia exposed lungs. Lung dry/wet weight was not altered indicating no edema following 24h hyperoxia. As seen from table 2, glucose-U-^{14}C incorporation into various lung lipids was not affected by acute hyperoxia (24h).

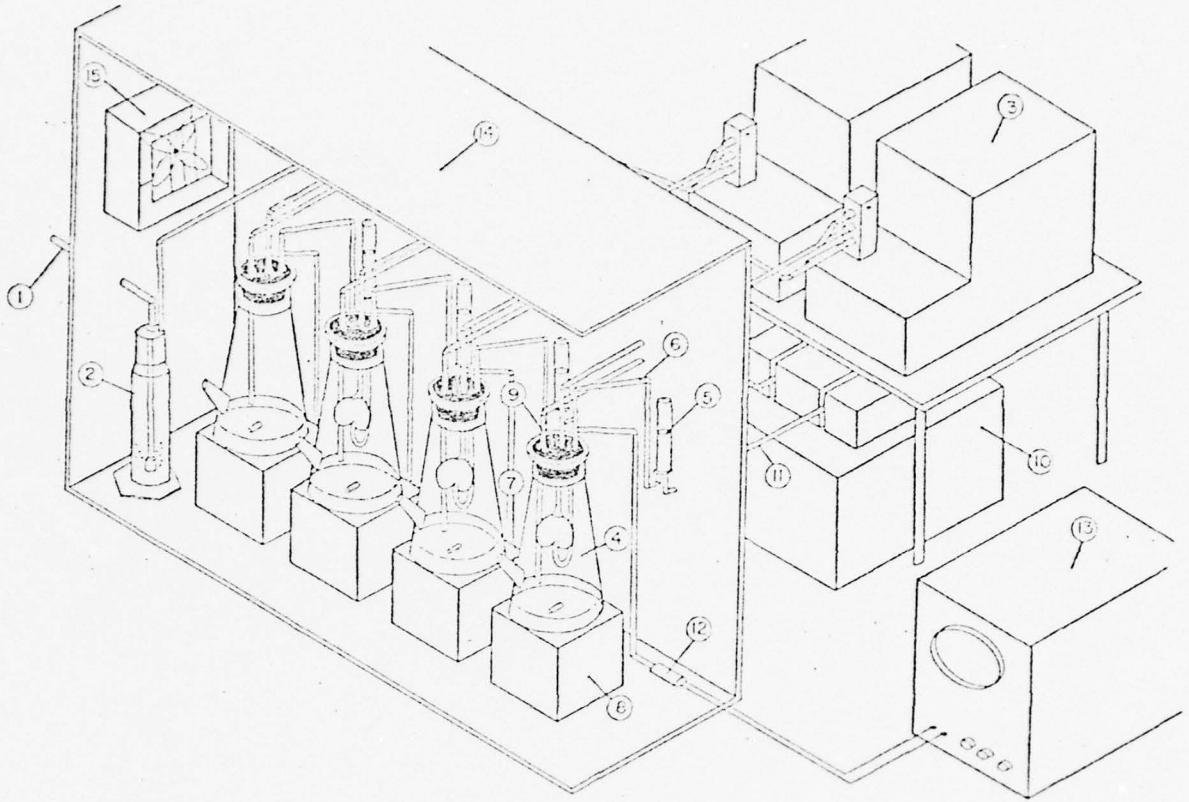

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Figure 1. SCHEMATIC DIAGRAM OF THE PERFUSION APPARATUS

Table 1. Influence of acute hyperoxia (24h) on substrate metabolism in the lung†

Measurement	Control (N = 6)	Hyperoxia (N = 7)
Glucose Uptake (μmoles · g dry lung^{-1} · h^{-1})	52.2±5.1	54.5±3.7
Lactate[a] (μmoles · g dry lung^{-1} · h^{-1})	125.7±10.4	161.0±10.0*
Pyruvate[a] (μmoles · g dry lung^{-1} · h^{-1})	12.8±0.6	7.6±0.8*
Lactate/Pyruvate	10.1±0.9	23.3±3.4*
Lung dry/wet weight (Postperfused)	0.16±0.012	0.15±0.008
pH	7.36±0.019	7.36±0.016
P_{O_2} (mmHg)	129±4	358±35
P_{CO_2} (mmHg)	35±1	35±2

† Values are averages ±SE. Hyperoxic rats were first exposed to 100% O_2 for 24h and then lungs removed and perfused for 1.5h. Flow rate in the perfused preparation for all lungs was 10ml · min^{-1}. Control lungs were ventilated with 21% O_2 - 5% CO_2 and hyperoxic perfused lungs were ventilated with 95% O_2 - 5% CO_2.

a Metabolites were measured from circulating medium

* Significant statistically from controls ($P < 0.05$).

Table 2. Influence of acute hyperoxia (24h) on glucose -U - ^{14}C incorporation into lung lipids†

Lipid	Control (N = 6)	Hyperoxia (N = 7)
Total lipid	2671±170	2690±170
Neutral lipid	494±40	481±30
Phospholipid (PL)	1793±89	1797±119
PL - fatty acid	153±29	149±14
PL - glyceride glycerol	1566±82	1707±98

† Values are means ±SE and expressed as nmoles · g dry lung^{-1} · h^{-1}. See table 1 legend for perfusion details.

These data indicate that although lipid synthesis is not immediately affected by 24h hyperoxia, the components of cellular activity are affected, particularly increased anerobic glycolysis and changes in pyruvate metabolism. How these changes in cellular activity are related to subsequent lung injury associated with hyperoxia exposure is not known at this time.

C. Influence of acute hypercapnia (1.5h) on substrate metabolism in the perfused lung.

In the next series of experiments the effect of 1.5h acute hypercapnia on lung metabolism was examined. Substrate concentration and the manner of perfusion were the same as described previously. Control lungs were ventilated with 21% O_2 - 5% CO_2, and lungs were made hypercapnic by ventilating them with 21% O_2 and 10% CO_2. P_{CO_2} for the hypercapnic lungs averaged 64.6 ± 1.6 during the 1.5h perfusion period (table 3). Acute hypercapnia did not alter glucose uptake or lactate production. Pyruvate expressed as $\mu moles \cdot g\ dry\ lung^{-1} \cdot h^{-1}$, however, showed a significant 26% decrease with a concomitant 36% increase in lactate/pyruvate ratio. Acute hypercapnia did not produce edema as seen from the dry/wet weight. Acute hypercapnia also did not alter glucose oxidation or incorporation into lung lipids (table 4). Moreover, the pattern of lipid synthesis was not affected.

D. Effect of acute hypocapnia (1.5h) on perfused lung.

Lungs were perfused in the same manner as above except experimental lungs were made hypocapnic by ventilating them with 21% O_2 - 3% CO_2. Hypocapnia did not significantly affect glucose uptake. Unlike high CO_2, hypocapnia resulted in a significant change in both lactate and pyruvate levels (table 5) without a marked change in lactate/pyruvate ratio. Lung dry/wet weight were not affected by acute hypocapnia indicating little edema. In table 6, glucose oxidation and incorporation into lung lipids are not affected by hypocapnia.

Table 3. Effect of acute hypercapnia (1.5h) on lung substrate metabolism†

Measurement	Control (N = 7)	Hypercapnia (N = 7)
Glucose (μmoles · g dry lung^{-1} · h^{-1})	48.4±4.9	36.0±6.7
Lactate[a] (μmoles · g dry lung^{-1} · h^{-1})	96.1±10.0	102.6±6.7
Pyruvate[a] (μmoles · g dry lung^{-1} · h^{-1})	9.5±0.5	7.0±0.5*
Lactate/Pyruvate	10.2±1.1	13.9±1.2*
Lung dry/wet weight	0.169±0.007	0.167±0.005
pH	7.33±0.02	7.30±0.02
P_{O_2} (mmHg)	125±1.5	130±1.6
P_{CO_2} (mmHg)	33±0.5	65±1.6

† Values are means ±SE. Lungs were perfused for 1.5h at a flow rate of 10ml· min^{-1}. Control lungs were ventilated with 21% O_2 - 5% CO_2 and hypercapnic lungs were ventilated with 21% O_2 - 10% CO_2.

[a] metabolite measured from perfusion medium.

* significant statistically from controls ($P < 0.05$)

Table 4. Effect of acute hypercapnia 1.5h on glucose -U - ^{14}C incorporation in the perfused lung†

Metabolite	Control (N = 7)	Hypercapnia (N = 7)
CO_2	1193±111	1087±239
Total lipid	3886±255	3629±170
Neutral lipid	926±91	874±58
Phospholipid (PL)	2494±157	2310±105
PL - fatty acid	422±39	374±37
PL - glyceride glycerol	2072±131	1935±93

† Values are means ±SE and are expressed as nmoles · g dry lung^{-1} · h^{-1}. See table 3 legend for perfusion details.

Table 5. Influence of acute hypocapnia (1.5h) on lung substrate metabolism†

Parameter	Control (N = 7)	Hypocapnia (N = 7)
Glucose (μmoles · g dry lung^{-1} · h^{-1})	48.4±4.9	41.3±7.0
Lactate[a] (μmoles · g dry lung^{-1} · h^{-1})	96.1±10.0	136.6±10.9*
Pyruvate[a] (μmoles · g dry lung^{-1} · h^{-1})	9.5±0.5	11.4±0.4*
Lactate/Pyruvate	10.2±1.1	12.0±0.8
pH	7.33±0.02	7.32±0.01
P_{O_2}	131.3±2.2	134.2±2.3
P_{CO_2}	32.7±0.5	22.8±0.3
lung dry/wet weight	0.169±0.007	0.171±0.003

† Values are means ±SE. Control lungs were ventilated with 21% O_2 - 5% CO_2 and hypocapnic lungs were ventilated 21% O_2 - 3% CO_2.

[a] obtained from perfusion medium

* significant statistically from controls ($P < 0.05$)

Table 6. Influence of 1.5h hypocapnia on glucose -U - ^{14}C incorporation in the perfused lung†

Metabolite	Control (N = 7)	Hypocapnia (N = 8)
CO_2	1193±111	1517±208
Total lipid	3886±255	3728±158
Neutral lipid	926±91	798±52
Phospholipids (PL)	2494±157	2435±116
PL - fatty acid	422±39	399±27
PL - glyceride glycerol	2072±131	2063±77

† Values are means ±SE and are expressed as nmoles·g dry lung^{-1} · h^{-1}. See table 5 legend for hypocapnic levels.

E. Influence of prior exposure to 24h hypercapnia on perfused lung

To examine the influence of prior exposure of hypercapnia on lung metabolism, rats were exposed to 12% CO_2 for 24h. Food and water were supplied ad libitum. Following 24h exposure, lungs were removed, perfused and ventilated with 21% O_2 - 10% CO_2 for 1.5h. Control lungs were ventilated with 21% O_2 - 5% CO_2. The perfusion medium was essentially the same as in part A. Twenty-four hour hypercapnia resulted in a significant ($P < 0.05$) 27% increase in adrenal weight (Table 7). Lungs fron these animals removed and subsequently perfused showed a 46% decrease in glucose uptake, 34% decrease in lactate production, and a 48% decrease in pyruvate levels. The ability of these lungs to incorporate U-^{14}C-glucose into various cellular components and metabolites is shown in Table 8. Prior exposure to 24h hypercapnia significantly depressed glucose incorporation into phospholipids (29%), and oxidation of glucose to CO_2 (39%).

These data indicate prior exposure to high CO_2 tensions markedly alters lung phospholipid synthesis and lung glucose oxidation.

Table 7. Effect of 24h hypercapnia on lung metabolism†

Measurement	Control (N=6)	Hypercapnia (N=6)
Adrenal weight (mg)	52.5 ± 3.4	66.6 ± 3.2*
Plasma glucose (mg/100ml)	193.8 ± 5.0	200.4 ± 6.3
Glucose uptake[a]	56.1 ± 3.1	29.9 ± 4.0*
Lactate production[a]	125.3 ± 11.1	83.3 ± 7.5*
Pyruvate[a]	7.1 ± 0.6	3.7 ± 0.3*
Lung dry:wet (Post perfused)	0.165 ± 0.009	0.158 ± 0.007

† Values are mean ±SE. Lungs from normal rats and rats exposed to 24h hypercapnia (12% CO_2) were removed, perfused and ventilated with 21% O_2-10% CO_2 for 1.5h. Control lungs were ventilated with 21% O_2-5% CO_2.

[a] Values denote levels in circulating medium from perfused lung, and are expressed as μmoles . g dry $lung^{-1}$. h^{-1}.

* Significant statistically from control ($P<0.05$)

Table 8. Influence of 24h hypercapnia on U-^{14}C-glucose into lung.†

Metabolite (nmoles ^{14}C-glucose converted/g dry lung/h)	Control (N=6)	Hypercapnia (N=6)
Total lipids	3318 ± 309	2439 ± 278
Neutral lipid	649 ± 59	522 ± 56
Phospholipid	2454 ± 219	1737 ± 186*
$^{14}CO_2$	1727 ± 84	1051 ± 83*
TCA - Soluble fraction	12,349 ± 1424	9042 ± 594
Protein	2185 ± 214	1727 ± 245
RNA - DNA	982 ± 56	842 ± 100

† Values are mean ±SE. See Table 7 legend for details.

* Statistically significant from control ($P<0.05$).

F. Substrate metabolism in the perfused lung: Influence of hypoxic-hypercapnic environment

The purpose of this study was to examine the effect of acute 24h hypoxia-hypercapnia on substrate metabolism. Male Long Evans Hooded rats were exposed to a 9% O_2 - 12% CO_2 gas mixture for 24h. Following the exposure, lungs were removed and placed on an isolated perfused organ apparatus (figure 1). All perfusions were carried out at 37°C. Lungs were perfused for 1.5 hours with a medium containing washed bovine red blood cells resuspended to a 15% hematocrit with Krebs Henseleit bicarbonate buffer containing 6g% dialyzed Pentex bovine serum albumin and 20 amino acids at physiological levels. Glucose and palmitate concentrations were 6mM and 0.4mM, respectively. pH was adjusted to 7.4 with 0.8M sodium carbonate. Substrate uptake was calculated as a product of initial and final concentration differences and perfusate volume. The amount of glucose degraded by blood cells was accounted for by circulating the medium in one set-up (blank) without a lung and measuring the substrate concentration changes attributed to the blood. All lungs were ventilated 50 cycles/min at tidal volume of 2.0ml. Blood flow was 10ml/min. Control lungs were ventilated with 21% O_2 - 5% CO_2. Hypoxic-hypercapnic lungs were ventilated with 9% O_2 - 12% CO_2. 10μCi of U-^{14}C-glucose and 50μCi 9, 10-^{3}H-palmitate were added as a single pulse.

From table 1, the 24h hypoxic-hypercapnic exposure resulted in significant changes in lung and body weights. Both the control and 24h exposed group were food deprived for the 24h period to separate anorexic from hypoxic-hypercapnic effects. Thus, the changes in body and organ weights appear to be due to the 24h exposure. Lung wet and dry weight

Table 1. Effect of 24h hypoxia-hypercapnia exposure on body and organ weights.†

Weight	Control	Hypoxia-Hypercapnia
Body weight, g		
initial	335.9±8.9	322.1±5.5
final	316.8±9.7	286.3±5.0*
Adrenal weight, g	0.0702±0.0042	0.0598±0.0040
Lung weight, g		
wet	1.3682±0.0607	1.6612±0.0807* (n=5)
dry	0.2304±0.0119	0.2921±0.0158* (n=5)
dry/wet	0.168±0.003	0.176±0.005 (n=5)

† Values are mean±SE with n=6/group unless specified otherwise. Both control and experimental groups were fasted for the 24h exposure period.

* Statistically significant from control ($P<0.05$).

showed a 21% and 27% increase, respectively. The change in wet and dry weight were proportional and yielded a normal lung dry/wet ratio. Prior 24h exposure also resulted in accelerated glucose uptake (67 percent) and a 30 percent increase in lactate production in IPL (table 2). Although pyruvate was not significantly affected, lactate/pyruvate ratio (L/P) did show a 50 percent increase following 24h exposure. In table 3, U- ^{14}C-glucose incorporation increased in both the neutral lipids - free fatty acids (NL) and phospholipids (PL) fractions with the largest percent increase appearing in the NL fraction (179 percent). Subsequent hydrolysis of the phospholipid fraction revealed that glucose incorporation was increased more in the phospholipid fatty acid moiety (110 percent) than in the glyceride-glycerol portion (31%). Therefore the percent distribution of the total lipid radioactivity was affected by 24h hypoxic-hypercapnic exposure. Glucose oxidation to CO_2, on the other hand, was not significantly influenced by 24h exposure to hypoxic-hypercapnic environment. Palmitate incorporation into lung lipids was also affected (table 4), although, unlike glucose incorporation which increased in all fractions, palmitate incorporation only increased significantly in the neutral lipid-free fatty acid fraction. These data indicate that a 24h hypoxic-hypercapnic environment markedly accelerates lipid synthesis in the lung. It is not known at this time if these changes in glucose uptake, lactate production, and lipid synthesis reflect adaptative processes to prevent lung injury or whether these changes constitute early stages of lung cell damage.

Table 2. Effect of 24h hypoxic-hypercapnic exposure on glucose uptake and lactate and pyruvate production in the isolated perfused rat lung. †

Metabolite	Control	Hypoxia-Hypercapnia
Glucose uptake	53.0±4.4	88.3±5.0* (n=5)
Lactate Production	139.9±10.7	181.8±13.3*
Pyruvate Production	7.7±0.6	6.7±0.5
Lactate/Pyruvate	18.3±0.8	27.5±2.2*

† Values are means±SE and expressed as μmoles · g dry lung^{-1}·h^{-1}. Lungs were perfused for 1.5h at a flow rate of 10ml/min and ventilated at 50 cycles/min (controls with 21% O_2 - 5% CO_2; experimental with 9% O_2 - 12% CO_2). N=6 unless specified otherwise. Both control and experimental groups were fasted for the 24h exposure period.

* Statistically significant from control ($P<0.05$)

Table 3. Influence of 24h hypoxic-hypercapnic exposure on U- ^{14}C-glucose incorporation in the IPL. †

Metabolite	Control	Hypoxia-Hypercapnia
Glucose Oxidized to CO_2	1398±166 (N=5)	1645±53 (N=5)
Glucose incorporated into:		
Total lipids	2493±116	4237±450*
Neutral lipid + FFA	356±32 (14)	992±94* (22)
Phospholipid (PL)	1672±75 (67)	2542±261*(60)
PL fatty-acid	324±17 (13)	683±88* (16)
PL glyceride-glycerol	1258±37 (50)	1643±156*(39)
PL non-saponifiable	45±2 (2)	119±16* (3)

† Values are mean±SE expressed as nmoles glucose incorporated · g dry lung $^{-1}$ · h^{-1}. N=6/group unless specified otherwise. Number in parenthesis is percent of total lipid radioactivity. See table 2 for perfusion details.

* Statistically significant from control ($P<0.05$).

Table 4. Influence of 24h hypoxia-hypercapnia on 9, 10-^{3}H palmitate incorporation in the IPL. †

Lipid	Control	Hypoxia-Hypercapnia
Total lipid	4508±86	5286±490
Neutral lipid + FFA	1079±68 (24)	1699±163* (32)
Phospholipid (PL)	2598±68 (58)	2617±192 (50)
PL fatty-acid	2087±49 (46)	2059±153 (39)
PL glyceride-glycerol	38±3 (1)	30±2 (1)
PL non-saponifiable	408±15 (9)	441±44 (8)

† Values are mean±SE expressed as nmoles glucose incorporated · g dry lung $^{-1}$ · h $^{-1}$. N=6/group. Number in parenthesis is percent of total lipid radioactivity. See table 2 for perfusion details.

* Statistically significant from controls ($P<0.05$).

Lung phospholipids are essential constituents of both the alveolar membrane and pulmonary surfactant - the surface-active material that coats the inner surface of alveoli and serves to lower and stabilize surface forces (1, 2). In vitro as well as in vivo studies have shown that the lung actively participates in lipid synthesis primarily directed toward the formation of phospholipids (1). Glucose and palmitate serve as major circulating substrates taken up by the perfused lung (3), and both play roles in phospholipid fatty acid (PLFA) synthesis. In the lung, circulating palmitate is preferentially incorporated over other serum fatty acids and is esterified into PLFA (4) while glucose can provide acetyl CoA for the synthesis of PLFA either by de novo synthesis or by chain elongation (5, 6).

The relative importance of the lungs ability to incorporate palmitate into PLFA versus the ability to synthesize PLFA from glucose has not been assessed. We, therefore, examined 9,10-^{3}H-palmitate incorporation into PLFA relative to U-^{14}C-glucose in the perfused lung.

METHODS

Male Long Evans hooded rats approximately 350g were given food (commercial rat chow) and water ad libitum. Rats were injected intraperitoneally (IP) with heparin (1 unit/g body weight). Fifteen minutes later, they were anesthetized with an IP injection of sodium pentobarbital (6 mg/kg) and bled via a carotid artery. Lungs were kept inflated and quickly excised, and perfused for 1.5h at a flow rate of 10 ml/min. Details of the perfusion apparatus and protocol for lung removal and cannulation as well as perfusion medium preparation have been described in detail elsewhere (3, 7). The basic procedure consisted of pumping blood through the lung via the pulmonary artery and allowing the medium to drip

from the left atrium into a reservoir flask for recirculation while the lungs were cyclically inflated-deflated at 100 cycles/min. Briefly, the perfusion medium consisted of washed bovine red blood cells resuspended to a 15% hematocrit with Krebs-Henseleit bicarbonate buffer - 5g% Pentex bovine serum albumin (Miles Laboratories). Glucose and palmitate (Sigma Chemical Co.) concentrations were 6 mM and .7 mM, respectively. The pH of the medium was adjusted to 7.4 with 0.8M sodium carbonate. Twenty μCi of U-^{14}C-glucose and 25 μCi of 9,10-^{3}H-palmitate (specific activity 42.5mCi/mMole) were added to the perfusion medium as a single pulse. The relative contribution of palmitate versus endogenous synthesis of PLFA from glucose was calculated as nmoles ^{3}H - Palmitate converted to PLFA/g dry lung/h / nmoles ^{14}C-Glucose converted to PLFA/g dry lung/h. The relative incorporation of palmitate and glucose into PLFA was also investigated during both 1.5h hypoxia and with 10^{-5} M corticosterone (Sigma Chemical Co.) added to the medium. Hypoxia was accomplished by ventilating perfused lungs with 5% O_2 - 5% CO_2 with the balance N_2. In another set of experiments 10^{-5} M corticosterone, which approximates physiologic concentrations (8), was added to the perfusion media. The solution was prepared by dissolving in ethanol (2.7 mg/ml).

Lipids were extracted and separated as previously described (9). Phospholipids were saponified by refluxing with 3.75% potassium hydroxide in methanol for 30 minutes at 67°C. The nonsaponifiable materials were extracted with light petroleum ether (b.p. 30-60°C). The remaining hydrolysate was acidified with 1N HCL and fatty acids extracted with light petroleum ether. The remaining aqueous portion contained phospholipid glycerol. Scintillation fluid for phospholipids, nonsaponifiables and phospholipid fatty acids was a toluene-based solution containing 4.0 g Omnifluor (Pilot chemical) per liter solution: The scintillant for phospholipid glycerol from hydrolysis was Omnifluor: Triton X-100 (Rohm & Haas) (2:1).

RESULTS

U-^{14}C-glucose incorporation into lung lipids was linear over the 1.5h period in the perfused lung (Fig. 1). Although not shown, over 65% of the amount of glucose incorporated into total lipids was utilized in phospholipid synthesis. The distribution between neutral and phospholipids remained unchanged indicating that the rate of glucose incorporation rapidly equilibrated and remained constant throughout the perfusion period. Other studies indicate labelled palmitate also rapidly equilibrates between the lipid classes (10).

Incorporation of labelled palmitate and glucose in the perfused lung under normal and hypoxic conditions, as well as in lungs perfused with 10^{-5} M corticosterone is shown in table 10. Over 80% of the palmitate converted into phospholipids appeared in PLFA. Some radioactivity appeared in the nonsaponifiable fraction and only trace amounts of H^3- label appeared in the phospholipid glycerol fraction. Approximately 15% of the glucose converted into phospholipids appeared in PLFA with remaining portion in the phospholipid glycerol. The ratio of palmitate utilized in PLFA synthesis to glucose utilized in PLFA was 8.5 indicating that for every mole of glucose utilized in PLFA synthesis, 8.5 moles of palmitate were utilized. Neither acute hypoxia nor the presence of 10^{-5} M corticosterone significantly effected ($P > 0.05$) the incorporation of glucose or palmitate into lung phospholipids during the 1.5h perfusion period. Moreover, the relative contribution of glucose and palmitate into PLFA was also unchanged.

Discussion

The lung has a considerable amount of lipoprotein lipase activity, and can readily extract fatty acids from chylomicrons or as low density lipoproteins both of which are available in large supply (11). Circulating

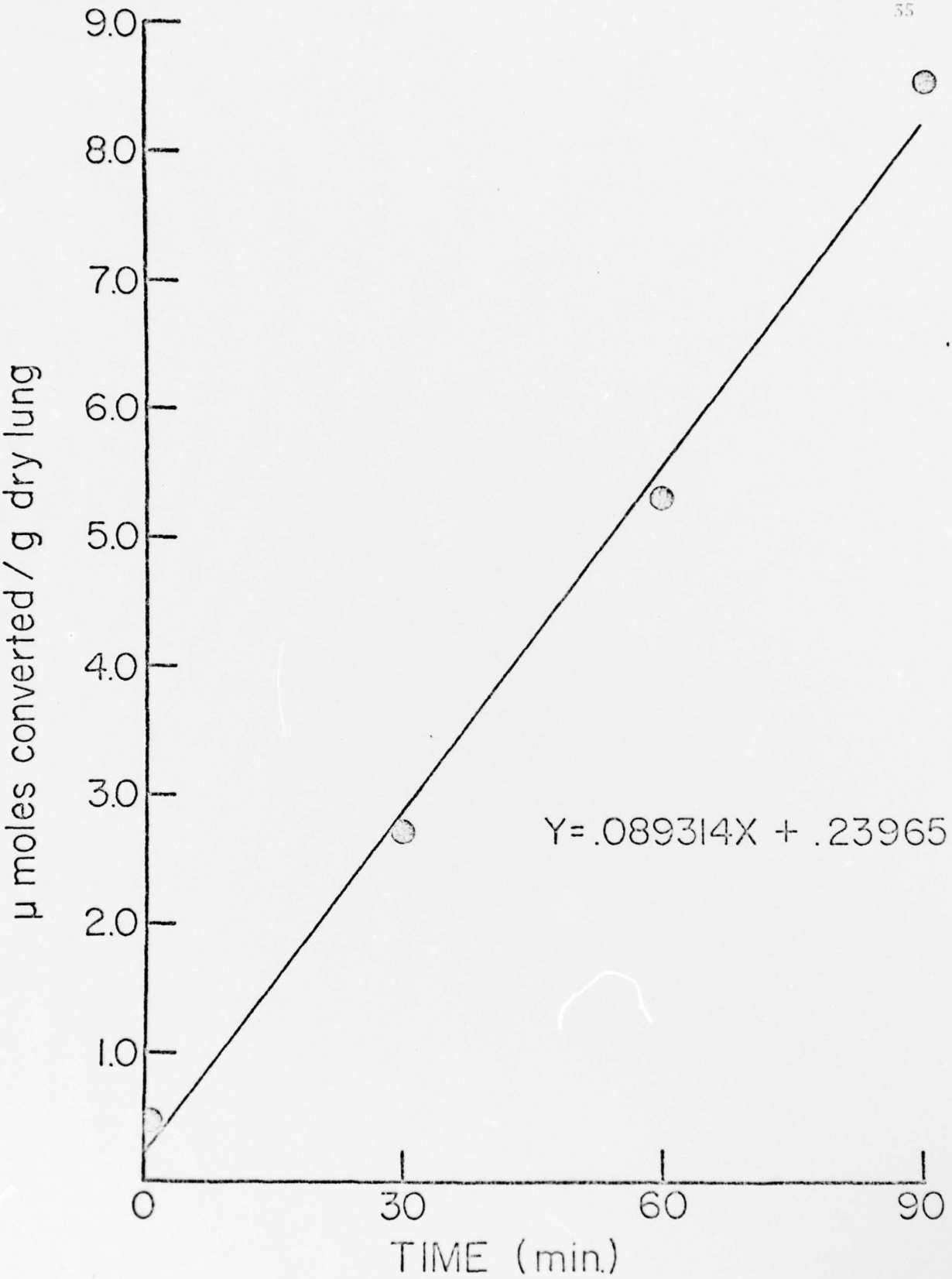

Figure 1. The conversion of 10 μCi of U-^{14}C-glucose into the total lipids of perfused lungs. All lungs were ventilated with 21% O_2 - 5% CO_2 - 74% N_2 (P_{O_2} = 121.3 ± 2.2 mmHg). Each point represents the average of 2 lungs.

Table 10. Relative incorporation of U - ^{14}C - glucose and 9, 10 - ^{3}H - palmitate into perfused lung lipids†

Lung lipid	nmoles of substrate converted/g dry lung/hr		
	Control (N=9)	Hypoxia[a] (N=8)	Corticosterone[b] (N=7)
Phospholipid			
^{3}H-palmitate	4421±282	4288±258	4226±315
^{14}C-glucose	2791±144	2658±154	2823±266
Phospholipid fatty acid (PLFA)			
^{3}H-palmitate	3562±224	3530±206	3276±255
^{14}C-glucose	408±49	409±22	361±35
Relative incorporation into			
PLFA[c]	8.5±0.84	8.7±0.44	9.3±0.80

†Values are averages ± S.E. Lungs were ventilated with 21% O_2 - 5% CO_2 unless otherwise specified.

[a]hypoxic lungs were ventilated with 5% O_2 - 5% CO_2. P_{O_2} in the perfusate averaged 34.9 ± 0.9 mmHg.

[b]perfusion medium contained 10^{-5} M corticosterone, dissolved in ethanol and added immediately before perfusion.

[c]expressed as $\frac{^{3}H\text{-palmitate incorporated}}{^{14}C\text{-glucose incorporated}}$

glucose is also actively taken up by the lung, even in the absence of insulin (12, 13). In the present study, both glucose and palmitate are readily incorporated into lung phospholipids, and in the presence of circulating palmitate, approximately 15% of glucose converted into phospholipids appeared in PLFA moeity. The remaining portion of glucose converted into phohpholipids appeared in the glyceride - glycerol moeity.

Recently, Wang and Meng (1) reported that in the perfused lung 20% of ^{14}C-phospholipid from glucose was in PLFA, which is slightly higher than seen in the present study. However, our circulating levels of palmitate were also higher, and the presence of palmitate is known to markedly affect the amount of glucose incorporated into PLFA (13). In a previous study with lung tissue slices, we have shown up to 40% of glucose radioactivity in the phospholipid appeared in PLFA when palmitate was absent from the media (9). High circulating glucose levels also increased the amount of glucose appearing in PLFA (14). From the foregoing observation circulating substrate levels affect the pattern of glucose incorporation into PLFA. As seen from table 1, when 0.7mM palmitate and 6mM glucose are present in the circulating medium, the ratio of palmitate utilized to glucose utilized in PLFA synthesis is 8.5, indicating approximately 8-9 moles of palmitate are incorporated for every mole of glucose. Expressed on a carbon basis, 96% of the PLFA carbons came from palmitate. From these data, it would seem that synthesis of lung PLFA is primarily from esterified palmitate and the synthesis from glucose, either de novo or by chain elongation, is of minor importance; thus emphasizing the significance of fatty acid uptake from circulating medium in perfused lung. No attempt was made in this study to differentiate chain elongation versus de novo synthesis. However, Sanders and Longmore (15) found by using mass spectrometric analysis of the products of U - ^{13}C glucose incorporation that

most of the endogenous synthesis of lung PLFA was from de novo synthesis. Although the relative importance of glucose incorporation into PLFA appears to be minor in the perfused lung under our conditions, it may be of physiological significance at different times in the intact animal where, for instance, short chain fatty acids are more available for elongation or substrate levels may vary (e.g. hyperglycemia).

Glucose and palmitate incorporation into lung phospholipids was not significantly affected in hypoxic perfused lung (P_{O_2} = 34.9 mmHg). This may be due, in part, to the fact that the magnitude of lipid synthesis in the lung is comparatively lower than that in adipose or liver tissue, and would not require large energy expenditures during a hypoxic stress. Moreover 1.5h hypoxic perfused lungs significantly increase glycolysis (unpublished observations) and resulting reducing equivalents may be sufficient to maintain lipid synthesis at these hypoxic levels as seen in table 1. Newman and Naimark (16) observed that palmitate incorporation into lung phospholipids was decreased *in vivo* when animals were made hypoxic by breathing 9% O_2 for 1 hr. The disparity between these findings and the present study are due more to *in vitro* versus *in vivo* effects than to differences in either manner of exposure or to the level of hypoxia. In vivo hypoxia alters other parameters such as blood flow and pH which are known to affect tissue response to O_2 tension (17, 18). In the perfused lung preparation such conditions are maintained and hence direct effects of hypoxia alone do not appear to be rate limiting for lipid synthesis.

The lung has been shown to have a large number of glucocorticoid receptor sites (19, 20). The function of these sites is not fully understood in the adult lung. One action of glucocorticoids in other lipogenic organs is to

block glucose entry into cells and depress lipid synthesis (21). The addition of 10^{-5} M corticosterone to the circulating medium did not significantly affect glucose incorporation into phospholipid in the perfused lung. The lack of an effect may be due, in part, to the fact that the 1.5h perfusion time was not sufficient to demonstrate a hormonal effect (22).

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H. Effect of acute hypoxia (24h) on lung cyclic nucleotides

Recent evidence suggests that adenosine 3', 5' - monophosphate (cAMP) and guanosine 3', 5' - monophosphate (cGMP) levels are potentially important in the regulation of lipid metabolism, contraction of smooth muscle in blood vessels and airways and tissue growth (1, 2, 3). Since hypoxia is known to affect all of these parameters (4-6), we investigated the alterations in cyclic nucleotide levels in lung following hypoxic exposure. Cyclic nucleotides were also examined in liver tissue to evaluate comparatively with lung, and with fasting, since food intake is substantially reduced in rats during hypoxic exposures (7).

METHODS

Male Long Evans Hooded rats weighing 250-300 g were exposed to 24h hypobaric hypoxia in an altitude chamber at 7,193 meters (23,600 feet: P_B = 280 *mmHg*). Rate of ascent and descent was 1000 feet $\cdot$ min^{-1}. The level of hypoxia was chosen to simulate clinical and altitude conditions as well as to correlate with previous experiments on the effects of hypoxia, performed in this laboratory (8). All animals received water ad libitum and the hypoxic group was food deprived for the 24h period. At the appropriate times animals were removed from the altitude chamber and killed within 15 min. by decapitation. Tissues were quickly removed, freeze clamped in liquid nitrogen and stored at -76°C until assayed. Forty mg of the frozen tissue were homogenized in 2ml of 6% trichloroacetic acid, centrifuged (2000xg) and the supernatant extracted with ethyl ether saturated with water. The aqueous portion was dried under nitrogen on a 60-70°C steam bath, resuspended in 2-4ml of 0.05 M sodium acetate (pH 6.2) and assayed for cyclic nucleotides using radioimmunoassay kits (9). Statistical analyses were carried out using Student's t tests (10).

No differences in lung dry:wet ratios were observed between hypoxic exposed animals and controls, indicating that little edema was present following acute hypoxic stress in rat lung. Lung and liver cyclic nucleotide levels are shown in Table I. In contrast to liver (fed ad lib condition) lung contains higher endogenous cAMP and cGMP levels. Acute hypoxia resulted in a significant ($P < 0.025$) decrease in cAMP while liver cAMP was unaltered. Cyclic GMP for both tissues was not affected by hypoxia. These data indicate that acute hypoxic stress has a selective action in decreasing lung cAMP.

Although a 24 hr fast did not markedly alter cyclic nucleotide levels, a 72 hr fast resulted in a 3-fold increase in lung cAMP while liver cAMP increased 8-fold. In both liver and lung, cGMP was unaltered. Although not shown, lung glycogen was unaffected by the 72 hr fast, whereas liver showed a 40-fold decrease in glycogen content, consistent with early studies (11, 8). These data emphasize the importance of the nutritional status of the animal on endogenous cyclic nucleotide levels and indicate that the preferential decrease in cAMP seen with hypoxic exposure cannot be explained as an effect due to anorexia. It is interesting to note that epinephrine will increase cAMP levels and that any stress, including starvation, may increase epinephrine and lead to elevated levels of cAMP. Thus, it is not clear at this time if the stress of food deprivation is the critical factor in stimulating cAMP levels. Nevertheless, nutritional stress appears to have a separate and distinct action on cAMP than seen with hypobaric hypoxic stress.

Table I. Cyclic nucleotide levels in lung and liver†.

CYCLIC NUCLEOTIDE	CONTROL (Fed Ad lib)	HYPOXIA (24 hr)	FASTED (24 hr)	FASTED (72 hr)
	pmoles/mg dry weight			
cAMP				
Lung	22.96±2.63 (n=6)	8.24±0.93* (n=7)	26.74±4.93 (n=10)	77.03±14.00* (n=4)
Liver	4.70±1.47 (n=6)	5.98±2.06 (n=4)	6.43±2.28 (n=4)	37.68±13.70* (n=4)
cGMP				
Lung	3.01±0.48 (n=3)	2.53±0.36 (n=7)	2.00±0.29 (n=9)	2.90±1.13 (n=4)
Liver	0.26±0.06 (n=3)	0.39±0.16 (n=4)	0.26±0.06 (n=4)	0.26±0.06 (n=4)
$\frac{cAMP}{cGMP}$				
Lung	7.62	3.25	13.37	26.56
Liver	18.07	15.33	24.73	144.92

† Values are mean±SE. Hypoxic animals were deprived of food and exposed to a simulated altitude of 7,195 meters

* Statistically significant from controls ($P < 0.025$)

The association of decreased cAMP levels or decreased cAMP - cGMP ratios with vasoconstriction and tissue growth indicate that alterations of cyclic nucleotide levels may be of functional significance in the hypoxic lung. Constriction of vascular smooth muscle associated with a decrease in cAMP/cGMP ratio has been reported by others (12). Hypoxia is known to cause pulmonary vasoconstriction. This raises the question of whether the decrease in cAMP observed in our investigation may serve as an underlying mechanism for the hypoxic pressor response (increase in mean pulmonary arterial pressure). Somewhat surprising was that cGMP was not elevated with hypoxia since prostaglandin (PG) $F_{2\alpha}$ - like substances have been shown to be released with hypoxia (13), and Vaughan and co-workers (14) have shown that cGMP plays an important role in regulating PG synthesis and release. Whether cGMP is linked to PG $F_{2\alpha}$ in lung remains to be determined.

An additional observation is that decreased levels of cAMP have been associated with tissue growth and elevation of cAMP associated with its suppression (15). In lung, where growth is increased with hypoxia, there is a decrease in cAMP, whereas in liver in which no such growth occurs, the cAMP levels are unchanged.

Since the lung is a heterogeneous organ comprised of at least 38 different cell types it should be noted that changes in tissue cyclic nucleotide levels may not totally reflect the response of any particular function or any specific cell type. Although the present study does not delineate the functional role of lung cyclic nucleotides it does provide fundamental information concerning changes in their levels during altered physiologic states.

In summary, rats, food deprived and exposed to hypobaric hypoxia (7,193 meters or 23,600 feet) for 24 hr, showed a significant decrease in lung adenosine 3', 5' - monophosphate (cAMP). Lung guanosine 3', 5' - monophosphate (cGMP) was unaltered as well as liver cAMP and cGMP. In contrast, rats fasted for 72 hr showed a significant 3-fold increase in lung cAMP and 8-fold increase in liver cAMP. Endogenous cGMP for both tissues was unchanged by a 72 hr fast. These data indicate that acute hypoxic stress has a selective action in decreasing lung cAMP and that this effect is not related to anorexia.

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III. PUBLICATIONS RELATED TO AIR FORCE GRANT NO. 2767

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